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# A phase 2 randomized controlled trial of a multicomponent meningococcal serogroup B vaccine, 4CMenB, in infants (II)

## Effects of variations of the OMV and protein content on immunogenicity and reactogenicity

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**Abbreviations:** 4CMenB, multicomponent meningococcal serogroup B vaccine; hSBA, serum bactericidal activity with human complement; DTaP, diphtheria-tetanus-acellular pertussis vaccine; DTaP-HBV-IPV/Hib, diphtheria-tetanus-acellular pertussis-inactivated poliovirus-hepatitis B with *Haemophilus influenzae* type b combination vaccine; PCV7, 7-valent pneumococcal conjugate vaccine; fHbp, factor H binding protein; NadA, Neisserial adhesin A; NZ OMV, Outer membrane vesicles from New Zealand serogroup B strain

The licensed meningococcal serogroup B vaccine, 4CMenB (Bexsero®), contains recombinant membrane proteins (rMenB) and outer membrane vesicles (OMV) of the New Zealand serogroup B strain. We investigated whether reducing the OMV and/or protein content influences 4CMenB immunogenicity and reactogenicity in healthy two month-old infants. Six formulations were studied: 4CMenB, rMenB with 0, ¼ or ½ the OMV dose in 4CMenB, a half-dose of 4CMenB or a prelicensure formulation of 4CMenB, as a 4-dose primary/booster series, concomitantly with routine vaccines (DTaP-HBV-IPV/Hib and 7-valent pneumococcal conjugate) at 2, 3, 4 and 12 months of age. Immunogenicity was assessed as serum bactericidal activity measured with human complement (hSBA) against indicator strains for Men B vaccine antigens before and after the 2,3,4-month series and 12-month dose. Parents recorded solicited reactions for 7 days after each vaccination, and any adverse events throughout the study period. All formulations elicited robust immune response against rMenB components at 5 months, there was some evidence of OMV and protein dose-dependence for Men B indicator strains tested. Titers waned up to the 12-month dose, which elicited further strong responses, which were still OMV and protein dose-dependent. Groups with no, or low-dose OMV displayed slightly lower reactogenicity profiles, but all formulations were generally well-tolerated, high fever was rare and transient, and only three transient SAEs were considered possibly vaccine-related. Decreasing or removing the OMV content reduced reactogenicity of 4CMenB to a certain extent, but had an unacceptable negative impact on the immunogenicity profile. **Trial:** Clinicaltrials.gov NCT00937521



## Introduction

Following successful introduction of polysaccharide conjugate vaccines against *Hemophilus influenzae* type b, pneumococcus and meningococcal serogroup C into infant immunisation schedules the outstanding remaining cause of infant meningitis and septicaemia is serogroup B. This meningococcal serogroup is unique among the major disease-causing forms (A, B, C, W and Y) in having a capsular polysaccharide that is unsuitable for vaccine development, being too similar to human neural tissue polysaccharides.[1,2] This has led to the use of novel technologies to develop vaccines to meet this unmet medical need, which has recently resulted in the approval of a multicomponent, recombinant protein serogroup B vaccine, 4CMenB (Bexsero®) in Europe and Australia.[3-6]

Prior to 4CMenB, the response to meningococcal serogroup B disease outbreaks was to develop vaccines based on outer membrane vesicles (OMV) of the clonal strains responsible for

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**Table 1.** Composition of different meningococcal serogroups B vaccine formulations

| | Quantity per dose | | | | |
|---|---|---|---|---|---|
| | **4CMenB *and* Prelicensure** | **rMenB** | **rMenB + ¼ OMV** | **rMenB + ½ OMV** | **½ 4CMenB** |
| **Antigens** | | | | | |
| fHbp-GNA2091 | 50 µg | 50 µg | 50 µg | 50 µg | 25 µg |
| NadA | 50 µg | 50 µg | 50 µg | 50 µg | 25 µg |
| NHBA-GNA1030 | 50 µg | 50 µg | 50 µg | 50 µg | 25 µg |
| OMV from *N. meningitidis* Strain NZ 98/254 | 25 µg | 0 | 6.25 µg | 12.5 µg | 12.5 µg |
| Other Ingredients | | | | | |
| Aluminum hydroxide | 1.5 mg | | | | 0.75 mg |
| NaCl | 3.12 mg | | | | 1.56 mg |
| Histidine | 10 mM | | | | 10 mM |
| Sucrose | 10 mg | | | | 5 mg |
| Water for injection | To 0.5 mL | | | | To 0.25 mL |

local outbreaks, most notably affecting Cuba, New Zealand and Norway.[7] However, while these vaccines were successful in dealing with the specific clonal outbreaks, their reliance on the main OMV antigenic factor, the porin protein (PorA), means they are too strain-specific to be suitable for a general serogroup B vaccine.[8,9] For this reason, the novel technologies of genetic sequencing and reverse vaccinology were used to identify antigenic proteins found on the surface of the serogroups B meningococcus with broad coverage across strains.[10] Three of these recombinant proteins, two prepared as fusion proteins with other antigen candidates, were formulated as an experimental vaccine, rMenB, which was tested in phase II studies in infants.[3,4] These studies also included formulations combining rMenB with OMV from the New Zealand outbreak strain, which were shown to have an enhanced immunogenicity profile.

In this phase II study, in order to guide future vaccine development strategies for MenB-containing vaccines, the final licensed formulation of the multicomponent, recombinant serogroup B vaccine, 4CMenB, containing rMenB and NZ OMV, was studied and compared with formulations containing variable quantities of the NZ OMV component and proteins, and with material used in the previous phase II studies (**Table 1**). Co-primary objectives were the safety and reactogenicity within 3 d of the first vaccination, and immune responses one month after the third. Additional arms designed to compare the reactogenicity of 4CMenB with polysaccharide protein conjugate meningococcal C (MenC) vaccine, and to the assess the influence of prophylactic paracetamol on this reactivity are reported in the accompanying paper.[11]

## Results

### Demographics

In the full study a total of 1507 infants were enrolled and randomly allocated to eight study groups, mean age 74.6 d, of whom 1137 were included in the six study groups described in this report, with baseline demographic characteristics presented in **Table 2** – data on the other participants are presented in the accompanying paper.[11] Groups were similar with respect to age, sex and ethnicity. There were 19 subject withdrawals during the primary series, distributed across the groups, principally due to withdrawal of consent (7) or being inappropriately enrolled (7). Two subjects were withdrawn due to adverse events, neither of which was assessed as vaccine-related. There were 23 withdrawals from the booster phase, the main reason being loss to follow up (18), but none due to adverse events (**Fig. 1**). All subjects exposed to vaccine and with data were assessed for safety and reactogenicity, but only per protocol analyses were performed for immunogenicity on those who provided blood samples at the 5 mo visit. Other than 71 subjects failing to provide a blood sample (6.2%), or 43 failing to receive all study vaccinations (3.8%), the main protocol violation was attending too late to provide blood samples outside of the protocol window (26–40 d after vaccination) by 21 subjects (1.8%). Three subjects were excluded due to unblinding issues, and one received the incorrect vaccination.

### Immunogenicity

Immunogenicity analyses were performed on the per-protocol populations, which included subjects who received all the relevant doses of vaccine correctly, provided at least one evaluable serum sample at the relevant time points, and had no major protocol violations. The major protocol violations leading to exclusion were the lack of blood samples, or providing the blood sample outside of the protocol window, after the third dose.

*4CMenB*

Immune responses were assessed as serum bactericidal activity with human complement (hSBA) against indicator strains selected to be sensitive to bactericidal antibodies from one of the three of the serogroup B antigens contained in 4CMenB – factor H binding protein (fHbp), Neisserial adhesin A (NadA) and NZ98/254 strain OMV (NZ OMV). These strains are not intended to be representative of the many hundreds of different strains found in clinical isolates. **Figure 2** shows the proportions of each group achieving an hSBA titer ≥ 5, which represents with 95% confidence the traditional threshold hSBA titer (≥ 4) which is considered an indicator for protection against meningococcal

**Table 2.** Study population demographics

| | Study groups | | | | | |
|---|---|---|---|---|---|---|
| | **4CMenB** | **rMenB** | **rMenB + ¼ OMV** | **rMenB + ½ OMV** | **½ 4CMenB** | **Prelicensure** |
| N (primary series) = | 188 | 188 | 192 | 190 | 191 | 188 |
| Age ± SD (days) | 74.0 ± 10.6 | 75.3 ± 8.8 | 74.4 ± 9.7 | 74.7 ± 9.7 | 74.8 ± 9.3 | 74.6 ± 9.0 |
| Male (%) | 53 | 49 | 57 | 52 | 59 | 51 |
| Weight ± SD (kg) | 5.5 ± 0.7 | 5.6 ± 0.7 | 5.6 ± 0.7 | 5.6 ± 0.7 | 5.6 ± 0.7 | 5.4 ± 0.7 |
| Height ± SD (cm) | 58.8 ± 2.6 | 59.2 ± 2.6 | 59.0 ± 2.7 | 59.4 ± 2.8 | 59.4 ± 2.6 | 59.0 ± 2.9 |
| Asian (%) | < 1 | 3 | 0 | 2 | < 1 | 1 |
| Black (%) | 0 | < 1 | 1 | 0 | 0 | 0 |
| Caucasian (%) | 96 | 89 | 93 | 92 | 94 | 94 |
| Hispanic (%) | 3 | 6 | 6 | 6 | 6 | 5 |
| Other (%) | < 1 | 2 | < 1 | < 1 | 0 | < 1 |
| N (booster dose) = | 155 | 169 | 169 | 163 | 168 | 165 |
| Age ± SD (days) | 386 ± 18 | 387 ± 21 | 385 ± 17 | 383 ± 16 | 386 ± 19 | 386 ± 19 |

infection.[6,12] At baseline there were low levels of hSBA in all groups, 2–5%, 3–8% and 0–1% of the groups having titers ≥ 5 against fHbp, NADA and NZ OMV indicator strains, respectively. One month after completion of the three dose primary series 99–100% of subjects had achieved this level against fHbp and NadA indicator strains, irrespective of the vaccine used.

Levels against fHbp waned up the 12-mo time-point when the booster dose restored hSBA titers ≥ 5 in 97–100% of subjects. In contrast, NadA titers were maintained ≥ 5 in 97–100% of subjects until the booster, after which all subjects (100%) had titers ≥ 5. Consideration of the fHbp geometric mean titers (GMT) does reveal some variations between formulations (**Table 3**). GMTs were consistent for both final and prelicensure 4CMenB formulations, and rMenB in the presence of ¼ or ½ doses of OMV (101–113), but the rMenB and half-dose 4CMenB groups had GMTs of 62 and 71, respectively. The difference with rMenB was maintained after the booster dose, 53 vs. 105–152, while the booster with half-dose 4CMenB achieved a GMT of 99.

GMTs against NadA were less affected by the absence or presence of OMV, the lowest GMT being achieved with a half-dose of 4CMenB, 316 vs. 371–534, while post-booster the lowest level was in the rMenB group, 730 vs. 983 for half-dose 4CMenB, and 1321–2238 for the other groups. However, as already noted, all subjects in all groups had an anti-NadA titer ≥ 5 after the booster dose.

Responses to the NZ98/254 strain, expressed as proportions with hSBA ≥ 5 or as GMTs, were much more variable, and consistent with the content of OMV in each formulation. As expected, there was limited response against NZ OMV in the rMenB group over the course of the study. There was a dose-dependent response against OMV in those groups with OMV-containing formulations; from a low background—at baseline GMTs ranged from 1.02–1.05 (0–1% with hSBA ≥ 5)—GMTs rose to 5.74, 7.81 and 10 (with 56%, 67% and 78% having a titer ≥ 5) after three vaccinations with ¼, ½ or full doses of OMV, respectively. The half-dose 4CMenB group response, GMT 6.66 (62% ≥ 5), was consistent with this pattern.

Waning of anti-OMV titers were more marked than against the other antigens, so only 3–12% had titers ≥ 5 at 12 mo. There was a booster response to the 4th vaccination at 12 mo, such that 78–89% had titers ≥ 5 at 13 mo (18% in the rMenB group), with the ¼ dose OMV and half-dose 4CMenB groups still displaying lower GMTs (11 and 14, respectively) than other groups (18–20).

At the time of this trial a suitable strain to unambiguously demonstrate response against the NHBA component antigen had not been identified, but post-primary responses to this antigen were measured with an ELISA (**Table 4**). Vaccine responses were clearly shown, with 175–214-fold increases in ELISA titers with a full dose of rMenB in the presence of OMV, but lower responses to rMenB alone (107) and the half-dose of 4CMenB (108).

*Routine vaccines – DTaP-HBV-IPV/Hib and pneumococcal antigens*

In view of the limited volume of serum available from infants and focused questions about a reduced dose OMV alternative vaccine, we did not measure responses to the concomitant routine vaccines—DTaP-HBV-IPV/Hib and pneumococcal conjugate—in all groups, but post-primary responses were measured in samples from the rMenB + ¼ OMV group for comparison with the 4CMenB group and a group who received meningococcal C conjugate rather than a Men B vaccine (**Tables S1 and S2**). These did not reveal any differences in response for any of the antigen responses as antibody levels or proportions achieving expected levels for the respective antigens.

**Reactogenicity**

One primary objective was a pairwise analysis of the group effect on safety and reactogenicity, specifically fever (defined as rectal temperature ≥ 38.5 °C) within 3 d of the first vaccination with the different formulations. This objective was met ($p < 0.05$) for the rMenB, ¼ dose OMV and half-dose 4CMenB groups compared with 4CMenB after the first vaccination. When examined after the second vaccination there was only a significant

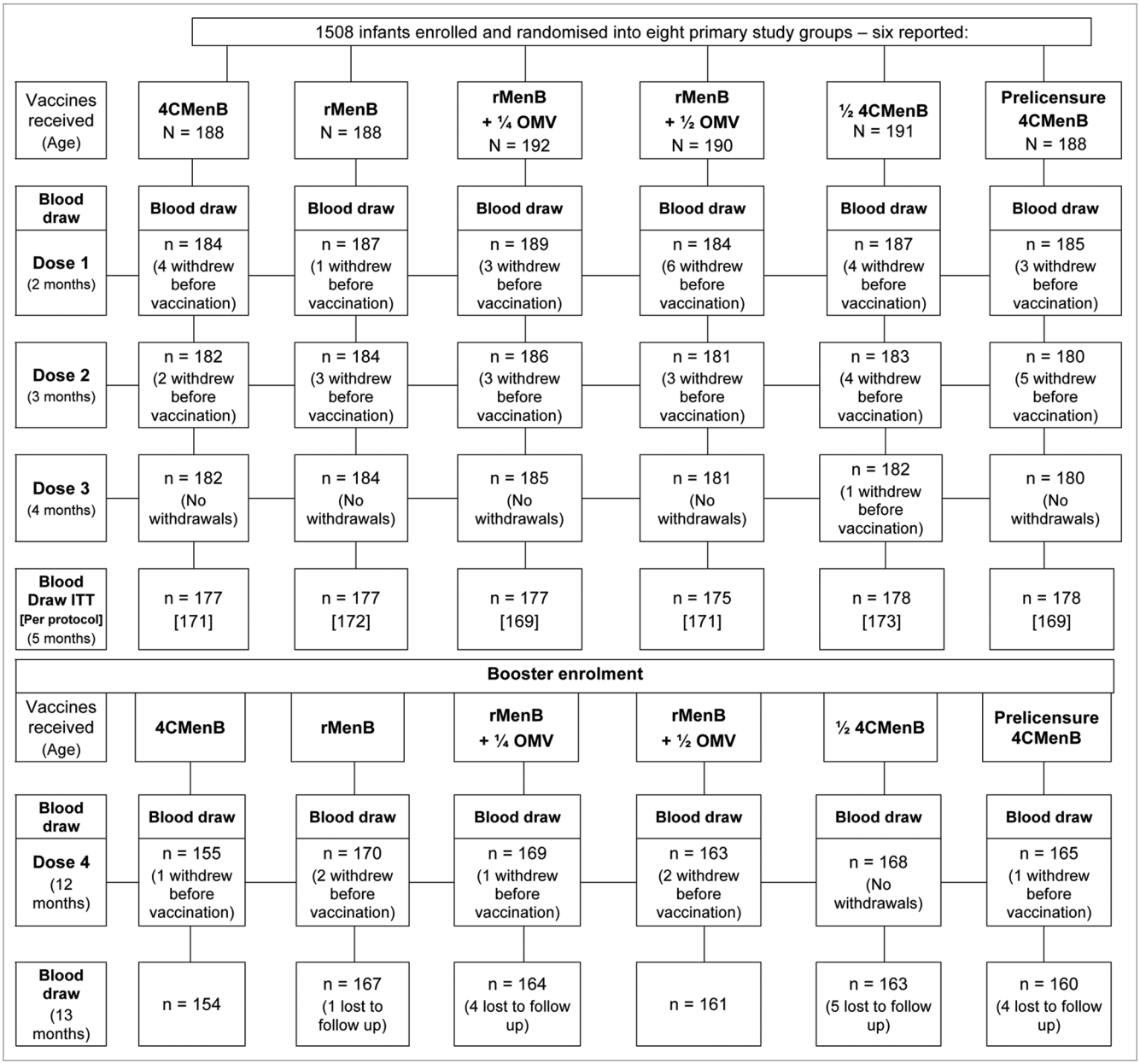


**Figure 1.** Study flowchart

effect for only the rMenB group ($p < 0.05$), and for the rMenB and ¼ dose OMV groups after the third.

As illustrated in **Figure 3**, a majority of subjects experienced at least one local or systemic reaction. The lowest rates were observed in the rMenB group, with no OMV, but there did not appear to be any meaningful decrease in the three groups who received lower doses of OMV (¼, ½ or the half-dose 4CMenB group).

*Local reactions*

Examining the individual local reactions at the Men B vaccine site revealed that OMV does contribute significantly to this reactogenicity (**Table S3**). The most frequent local reaction was tenderness at the injection site, which occurred in 53–67% of those receiving their first dose of OMV-containing vaccine, but in only 29% of the rMenB group (a similar rate to those observed for MenC vaccination reported in the part of the study[11]). The rMenB group also had a lower rate of the other local reactions, which were equally frequent in the other groups.

Local reaction rates were similar across doses 1, 2 and 3, but were highest to the dose 4 at 12 mo. As with the first dose, rates were consistently lower in the rMenB group after doses 2–4. Rates of local reactions to OMV-containing formulations were similar to those reported at the DTaP-IPV-HBV/Hib injection site, these rates being unaffected by the differences in the formulations of the Men B vaccine (data not shown).

*Systemic reactions*

Although temperature ≥ 38.5 °C was common in the 7-d period after vaccination, occurring in approximately half the subjects after the first dose, there was only one case of fever ≥ 40.5 °C throughout the study, following a booster dose in the half-dose 4CMenB group (**Table 5**). Fever rates were lower after the third vaccinations, but occurred at similar levels to the first dose after the booster. There was clear evidence of a contribution of OMV to fever in the different groups; lowest rates of fever after all doses occurred in the rMenB group, 13% after dose 1 compared with 33–53% in the groups with OMV-containing formulations. The equivalent rates were 20% and 41–50% for rMenB and the other groups after the second dose, 9% and 21–30% after the third dose, and 26% and 38–53% after the booster dose. Generally, rates were similar all in all groups who received OMV, irrespective of the OMV dose itself.

Cases of medically attended fever were relatively infrequent after each dose. Rates of antipyretic use were congruent with the rates of fever, in line with treatment of fever or other local or systemic reactions. The rates were also lower in the rMenB group (**Table 5**).

Other solicited systemic reactions (**Table S5**) also occurred at lower rates in the rMenB group compared with the groups with OMV-containing formulations. After the first dose with OMV-containing formulations, the most frequent reactions were irritability (69–73%), sleepiness (64–73%), unusual crying (52–60%), and change in eating habits (42–50%), compared with 54%, 52%, 33% and 28% in the rMenB group, respectively. Rates of most reactions were lower with subsequent doses, irritability being a notable exception, but rates in the rMenB groups were always the lowest.

*Spontaneously reported AEs*

Overall, rates of AEs were similar all groups with 79–85% of the subjects in all groups having a spontaneous adverse event reported during the primary series (**Table S6**). When assessed by the investigators rates of AEs at least possibly related to vaccination fell to 29% in the rMenB group, 27% in the half-dose 4CMenB group and 40–41% in the others. Rates after the booster vaccinations were 66–71% for any, and 14–28% for possibly related. The possibly related AEs were generally injection site reactions that extended beyond 7 d and by sponsor convention were considered therefore unsolicited reactions. Again, rMenB and half-dose 4CMenB groups had the lowest rates (14%). SAEs were reported in 6–11% of each group during the primary series, 3–9% after the booster, but only three SAEs were considered by the investigator to be at least possibly related to study vaccination; a case of vomiting 6 d after the third dose of rMenB+¼ OMV which necessitated a three-day hospitalisation, somnolence on days 1–3 after a first dose of rMenB+½ OMV, and a 6 cm erythema which developed immediately after a booster dose of rMenB+¼ OMV. All these cases resolved.

Of four seizures (febrile and afebrile) reported in three subjects during the study, none were temporally associated with vaccination, and all were considered by the investigator to be unrelated to the study vaccinations. One case of a febrile seizure occurred 127 d after a third dose of 4CMenB administered with routine vaccines. In the rMenB+½ OMV group one subject had a febrile seizure 62 d after a MenC vaccination, and 147 d after the booster vaccination with rMenB+½ OMV and routine vaccines. Another subject in the rMenB+½ OMV group had afebrile seizures at 98 and 181 d after the third doses of rMenB+½ OMV with routine vaccines.

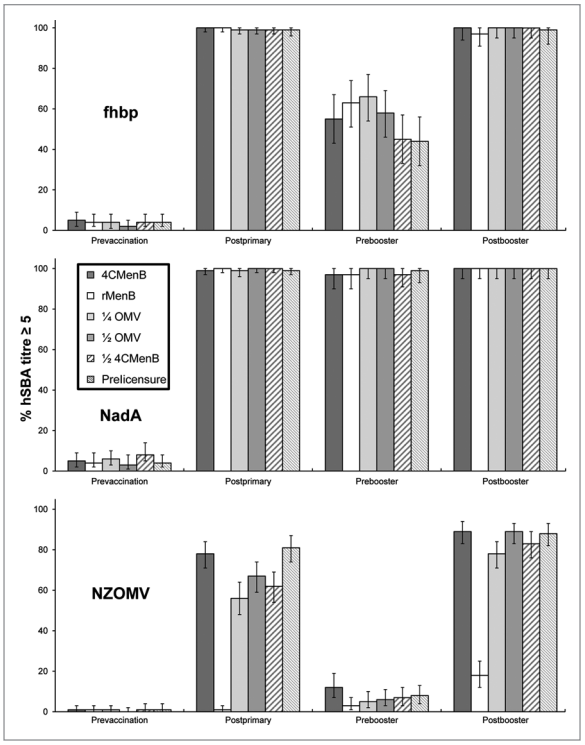


**Figure 2.** Percentages of subjects in each group (with 95% CI bars) with hSBA titers ≥ 5 against three indicator serogroup B strains for fHbp, NadA and NZ OMV antigens before vaccination, one month after the third dose, before the booster dose at 12 mo and one month after the booster dose in the six study groups.

## Discussion

In regions such as Europe, where routine infant immunisation includes polysaccharide-protein conjugate vaccines against *Hemophilus influenzae* type b, pneumococcus and meningococcal serogroup C, the major cause of infant meningitis and septicaemia is serogroup B meningococcus. A vaccine with broad coverage against different meningococcal serogroup B strains has remained elusive for many years as the polysaccharide-protein conjugate methodology is impractical due to the nature of the serogroup B capsule characteristics.[1] Clonal strain-specific vaccines based on outer membrane vesicles (OMV) of outbreak strains, in which the main antigenic contribution is from the variable PorA component, provided specific protection against regional strains, but not broad coverage.[7] Such a vaccine was only finally realized with the application of reverse vaccinology to identify alternative protein antigens, three of which are included

**Table 3.** hSBA Geometric mean titers (GMT) against indicator strains for serogroup B antigens in all study groups, before and one month after primary and booster vaccinations

| Antigen | Time-point | 4CMenB | rMenB | rMenB + ¼ OMV | rMenB + ½ OMV | ½ 4CMenB | Prelicensure |
|---|---|---|---|---|---|---|---|
| fHbp | Baseline | **1.25** (1.14-1.37) n = 166 | **1.19** (1.08-1.3) n = 170 | **1.2** (1.1-1.32) n = 168 | **1.12** (1.03-1.23) n = 171 | **1.31** (1.19-1.43) n = 169 | **1.2** (1.09-1.31) n = 168 |
| | Post-3rd dose | **101** (90-113) n = 170 | **62** (56–70) n = 166 | **113** (101-126) n = 166 | **112** (101–126) n = 170 | **71** (64–80) n = 169 | **102** (92-114) n = 167 |
| | Pre-booster | **4.94** (3.76–6.5) n = 69 | **5.44** (4.19–7.06) n = 78 | **5.72** (4.41–7.42) n = 74 | **5.22** (4.03–6.76) n = 78 | **3.96** (3.02–5.18) n = 71 | **3.76** (2.87–4.94) n = 71 |
| | Post-booster | **120** (95-150) n = 65 | **53** (43–66) n = 75 | **118** (95-146) n = 70 | **152** (122-189) n = 73 | **99** (79-122) n = 76 | **105** (84-131) n = 71 |
| NadA | Baseline | **1.18** (1.07-1.3) n = 162 | **1.13** (1.03-1.25) n = 161 | **1.12** (1.02-1.34) n = 161 | **1.09** (0.99-1.2) n = 162 | **1.3** (1.18-1.43) n = 166 | **1.16** (1.05-1.27) n = 166 |
| | Post-3rd dose | **396** (348–450) n = 165 | **389** (342–443) n = 166 | **534** (469–608) n = 161 | **503** (442–572) n = 159 | **316** (278-360) n = 165 | **371** (326–422) n = 161 |
| | Pre-booster | **69** (53–88) n = 71 | **74** (57–94) n = 72 | **111** (87-141) n = 80 | **91** (71-116) n = 76 | **54** (42–68) n = 77 | **64** (50–81) n = 78 |
| | Post-booster | **1950** (1573-2417) n = 73 | **730** (590–903) n = 72 | **2238** (1820-2751) n = 79 | **1819** (1478-2238) n = 77 | **983** (801-1205) n = 76 | **1321** (1074-1624) n = 74 |
| NZ OMV | Baseline | **1.02** (0.99-1.06) n = 170 | **1.05** (0.9–1.23) n = 168 | **1.03** (1–1.06) n = 171 | **1.02** (0.99–1.05) n = 174 | **1.03** (1–1.06) n = 171 | **1.04** (1.01–1.08) n = 173 |
| | Post-3rd dose | **10** (8.59-12) n = 171 | **1.03** (0.87–1.21) n = 162 | **5.74** (4.92–6.71) n = 169 | **7.81** (6.69–9.12) n = 172 | **6.66** (5.71–7.77) n = 172 | **11** (9.16–13) n = 169 |
| | Pre-booster | **1.6** (1.43-1.8) n = 141 | **1.11** (0.99–1.24) n = 150 | **1.23** (1.1–1.37) n = 155 | **1.28** (1.15–1.43) n = 155 | **1.35** (1.21–1.5) n = 150 | **1.41** (1.26–1.57) n = 153 |
| | Post-booster | **20** (16-24) n = 138 | **1.67** (1.38–2.03) n = 149 | **11** (9.07–13) n = 150 | **18** (15–22) n = 152 | **14** (12–17) n = 152 | **20** (16–24) n = 146 |

in the 4CMenB formulation, which has recently been licensed in Europe and Australia.

Early phase II studies in infants of 4CMenB investigated the recombinant proteins alone (rMenB) or with OMV (4CMenB) from the New Zealand outbreak strain (NZ98/254) containing PorA 1.4.[3,4] These indicated a beneficial effect of the OMV, not only in terms of extending coverage with an immune response against PorA, but also enhanced responses to the other protein antigens and limited increase in reactogenicity. For these reasons, the selected formulation for subsequent phase II and III studies included NZ98/254 OMV. However, inclusion of 4CMenB in routine infant vaccination schedules, with concomitant administration with DTaP-HBV-IPV/Hib and pneumococcal conjugate vaccines, was associated with an incremental increase in reactogenicity, particularly fever and injection site tenderness.[4-6] As increased fever could compromise the acceptability of 4CMenB in infant vaccination schedules, we performed a study to investigate formulations with reduced OMV content to inform future vaccine development, as well as the use of prophylactic paracetamol as reported in the companion paper.[11]

As previously reported,[3,4] the rMenB vaccine formulation containing only recombinant proteins without OMV displayed slightly lower rates of local and systemic reactogenicity, including fever, and were consistent with the rates reported for MenC vaccination in that part of the study reported in the complementary paper.[11] However, omission of OMV decreased the height and breadth of coverage in the limited strain testing available. With the inclusion of the OMV, the incremental increase in overall reactogenicity was well tolerated, and there were no vaccine-related serious adverse events (SAE) and withdrawal rates were similar in all groups. Most reactions were generally mild and transient, and local rates were similar to those of the routinely used infant

**Table 4.** Geometric mean concentrations (GMCs) (95% CI) of ELISA antibodies (EL.U/mL) against the NHBA component

| | ELISA GMC (EL.U/mL) (95% CI) | | | | | |
|---|---|---|---|---|---|---|
| | **4CMenB** | **rMenB** | **rMenB + ¼ OMV** | **rMenB + ½ OMV** | **½ 4CMenB** | **Prelicensure** |
| Baseline | **21** (19–22) n = 172 | **21** (19–22) n = 174 | **21** (20–22) n = 173 | **22** (21–23) n = 173 | **22** (21–24) n = 172 | **22** (20–23) n = 174 |
| Post-3rd dose | **3562** (3161–4013) n = 173 | **2170** (1923–2447) n = 171 | **4395** (3897–4957) n = 169 | **4106** (3642–4629) n = 172 | **2373** (2107–2672) n = 174 | **3755** (3328–4236) n = 169 |
| GMR of Post-3rd to baseline | **178** (156–204) n = 165 | **107** (93–123) n = 165 | **214** (186–245) n = 161 | **194** (169–222) n = 167 | **108** (95–124) n = 168 | **175** (153–200) n = 167 |

vaccines. Anticipation of the increase in fever through informing the parents, or the prophylactic use of paracetamol as reported in our companion report, suggest that this incremental increase would not influence the acceptance of the vaccine. Although the numbers are limited in this study, rates of medically attended fever were similar in all groups, even when comparing those with full doses of OMV with the rMenB group with no OMV and generally lower fever rates.

The novel vaccine 4CMenB is designed to offer a broad protection against as many serogroup B strains as possible. Because of the nature of meningococcal B strains, which display great variability in the proteins they can and actually do express, it is not possible to use a single antigen to cover them all. By using multiple antigens 4CMenB offers broad protection, which can only be assessed using techniques such as the meningococcal antigen typing system (MATS) that employs a sandwich ELISA to predict the likelihood that individual strains will be killed in the hSBA.[13,14] Applying MATS to over 1000 strains isolated across eight European countries predicted that the current 4CMenB formulation would cover 78% (95% CI 63–90) of all strains, and that over half of the strains would be targeted by more than one antigen.[15] This is probably a conservative estimate as the MATS technique does not allow any estimate of synergies due to different antigens, nor does it take into account potential immune responses for minor components such as additional non-PorA proteins in the OMV.

This study suggests that with removal of the OMV component of 4CMenB, an unsatisfactory decrease in immunogenicity is observed and decreases of either protein or OMV levels will result in decline of overall immunogenicity without a meaningful benefit in the reactogenicity profile. The results support the current formulation of 4CMenB, which is generally well tolerated when administered with routine vaccines, elicits robust immune responses against all components, some of which display good persistence at least until at least 40 mo of age.[16,17] With the results of our companion report, we suggest that where there are concerns about the transient reactogenicity of the vaccine, an option to employ prophylactic paracetamol can be considered and the vaccine can be safely incorporated into current infant immunisation schedules, and so provide protection against the last major remaining cause of infant meningitis and septicaemia.

## Methods

### Study design and participants

This was a phase II, randomized, controlled, multicenter study conducted in multiple centers in the Czech Republic, Italy, Hungary, Chile and Argentina between July 2009 and November 2010 to assess the effects of six different formulations of a meningococcal serogroup B vaccine on safety and immunogenicity profiles. The study was designed according to Good Clinical Practice and the Declaration of Helsinki, with approval of the protocol by ethics committees of participating centers and prior to enrolment, written informed consent from the parents or guardian of each participant.

The study also included two other groups designed to assess the impact of the final formulation of the investigational serogroup B meningococcal vaccine, 4CMenB, on fever rates compared with meningococcal C conjugate vaccine as control, and the effect of prophylactic administration of paracetamol on these rates. These arms are reported in the accompanying article.[11]

Study participants were healthy infants aged 2 mo (55–89 d, inclusive) at the time of enrolment. Infants were excluded if they had been in receipt of any antipyretic medication in the previous 6 h or if they had a contraindication to paracetamol treatment. Other exclusion criteria were any history of disease caused by *N. meningitidis* or intimate exposure to an individual with laboratory confirmed *N. meningitidis*, or prior receipt of any meningococcal B or C, diphtheria, tetanus, pertussis, polio, Hib, or pneumococcal vaccines; significant acute or chronic infection within the previous 7 d or temperature ≥ 38 °C within the previous day; oral or parenteral antibiotic treatment in the 7 d prior to the scheduled blood draw; impairment/alteration of the immune system; receipt of blood, blood products and/or plasma derivatives or any parenteral immunoglobulin preparation; receipt of, or intent to immunize with any other licensed vaccine(s) (with the exception of rotavirus vaccines), from 28 d prior to enrolment to 28 d after the last study vaccination and; participation in another clinical trial.

### Vaccines

The licensed meningococcal serogroup B vaccine formulation, 4CMenB (Bexsero®; Novartis Vaccines) was supplied in 0.5mL doses in pre-filled syringes, containing 50µg each of three recombinant protein antigens - factor H binding protein (fHbp) as a

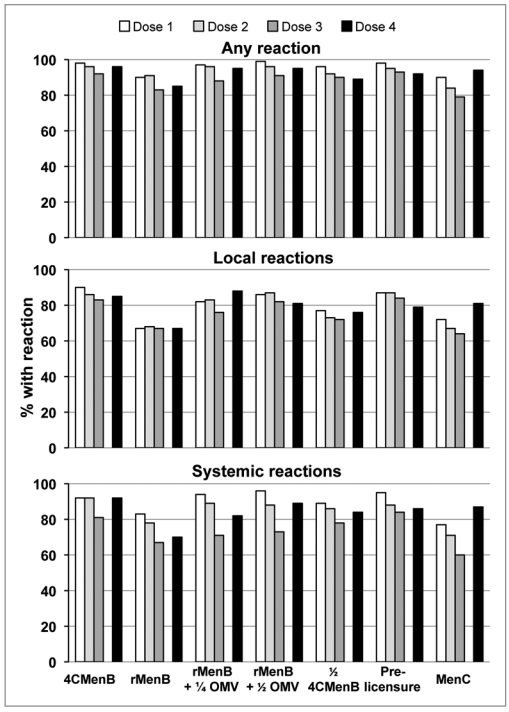


**Figure 3.** Percentages of subjects in each group with at least one report of any reaction, a local reaction or a systemic reaction after each vaccination in the six study groups

fusion protein with another meningococcal protein GNA2091, Neisserial adhesin A (NadA), and *Neisseria* heparin binding antigen (NHBA) also as a fusion protein with protein GNA1030, together with 25μg OMV from *N. meningitidis* strain NZ98/254 and 0.5 mg of $Al^{3+}$ in the form of $Al(OH)_3$ in 10mM histidine/saline buffer (**Table 1**).[4-6] The prelicensure formulation had the same composition prepared from pre-licensure materials and the experimental formulations contained the same components in the variable combinations and quantities described in **Table 1**. Concomitantly administered routine infant vaccines were DTaP-HBV-IPV/Hib and 7-valent pneumococcal conjugate vaccine (PCV7, Prevenar®; Wyeth, Philadelphia, USA). Composition and use is described in the companion paper.[11]

Four doses of each of the three vaccines were administered by intramuscular injection at 2, 3, 4 and 12 mo of age; MenB vaccines were administered intramuscularly into the right thigh, and DTaP-HBV-IPV/Hib and PCV7 vaccines were both administered intramuscularly into the left thigh at least 2.5 cm apart.

**Randomization and Procedures**

Local investigators enrolled infants who were randomly assigned using a web-based randomization system (1:1:1:1:1:1:1:1 ratio, block size = 8) supplied by the study sponsor into one of eight study groups, six of which are reported here. The other two groups, as well as the 4CMenB group, are reported in the companion paper.[11]

Blood samples were collected from all subjects prior to the first dose and 30 d following the third vaccination for immunogenicity assessments. Sera were analyzed using validated methods at the Novartis laboratory (Clinical Laboratory Science, Novartis Vaccines, Marburg, Germany) or a designated contract laboratory. Immune responses to 4CMenB were assessed as serum bactericidal activity using human complement (hSBA) against three *N.meningitidis* test strains H44/76, 5/99 and NZ98/254, specific for three of the vaccine components (fHbp, NadA and NZ OMV, respectively), using a titer ≥ 5 as the lower limit of quantitation (LLQ) which represents with 95% confidence the traditional threshold hSBA titer (≥ 4) which is considered an indicator for protection.[12] As no suitable strain for NHBA responses has been identified at the time of the study, responses to NHBA were assayed using an NHBA-specific ELISA at baseline and post-dose 3. Immune responses to routine vaccines, assessed by standard ELISA or neutralization test methods, are described in the companion paper.[11]

**Safety and Reactogenicity**

Subjects were observed for a minimum of 30 min after receipt of each vaccine dose to monitor for immediate adverse reactions and parents then recorded solicited reactions for 7 d post-vaccination on diary cards. Solicited local reactions (i.e., injection site tenderness, erythema, induration and swelling) and systemic reactions (i.e., change in eating habits, sleepiness, vomiting, diarrhea, irritability, unusual crying, and urticarial or other rash). Other indicators of reactogenicity were body temperature measured as rectal temperature with supplied study thermometers, (fever defined as rectal temperature ≥ 38.5 °C), and the use of antipyretic medication. Unsolicited adverse events (AEs) and serious adverse events (SAEs) were recorded throughout the study. Assessments of the causal relationship of adverse events to the vaccination were made by the investigator or study physician and were classified as not related, possibly related, or probably related.

**Statistical analyses**

Immunogenicity analyses were run on the per-protocol population, which consisted of subjects who received all the relevant doses of vaccine correctly, provided at least one evaluable serum sample at the relevant time points, and had no major protocol violations; reactogenicity was analyzed for all subjects exposed to study vaccines. The percentages of subjects in each group with hSBA titers ≥ 5 against *N. meningitidis* serogroup B strains H44/76, 5/99 and NZ 98/254, and associated two-sided 95% Clopper-Pearson Confidence Intervals (CIs) were computed at baseline, at month 12, and one month after the third and fourth vaccinations. Safety and tolerability data were summarized by vaccine group providing the percentage of subjects reporting an event. Only descriptive analyses are presented.

### Conflicts of interest

SE has received research grants from Crucell, GSK and Pfizer, and honoraria for consultancy work with Novartis and GSK. RP has received research grants and honoraria from Novartis, GSK, Pfizer, Baxter and Sanofi Pasteur. GVZ received study fees from Novartis. FX, MB, PD and DT are full-time employees of Novartis Vaccines and Diagnostics.

**Table 5.** Percentages of subjects per group with fever, cases of medically attended fever and rates of antipyretic use during the 7 d after each vaccination

| | | | 4CMenB | rMenB | rMenB + ¼ OMV | rMenB + ½ OMV | ½ 4CMenB | Prelicensure | MenC |
|---|---|---|---|---|---|---|---|---|---|
| Dose 1 | | | **n = 182** | **n = 184** | **n = 186** | **n = 180** | **n = 182** | **n = 180** | **n = 177** |
| | Fever (%) | ≥ 38.5°– < 39 °C | 31 | 10 | 27 | 36 | 22 | 25 | 11 |
| | | 39.0°– < 39.5 °C | 15 | 2 | 10 | 14 | 10 | 13 | 1 |
| | | 39.5°– < 40.0 °C | 5 | 1 | 3 | 1 | 0 | 4 | 0 |
| | | ≥ 40.0 °C | 1 | 0 | 0 | 0 | 1 | 1 | 0 |
| | Medically attended fever (n) | | 4 | 1 | 0 | 3 | 1 | 2 | 2 |
| | Antipyretic use (%) | | 56 | 20 | 49 | 52 | 41 | 53 | 32 |
| Dose 2 | | | **n = 182** | **n = 184** | **n = 186** | **n = 180** | **n = 182** | **n = 180** | **n = 177** |
| | Fever (%) | ≥ 38.5°– < 39 °C | 34 | 16 | 28 | 32 | 29 | 28 | 13 |
| | | 39.0°– < 39.5 °C | 12 | 3 | 11 | 10 | 8 | 16 | 3 |
| | | 39.5°– < 40.0 °C | 4 | 1 | 2 | 3 | 3 | 6 | < 1 |
| | | ≥ 40.0 °C | 0 | 0 | 1 | 1 | 0 | 0 | < 1 |
| | Medically attended fever (n) | | 1 | 0 | 1 | 0 | 6 | 1 | 1 |
| | Antipyretic use (%) | | 55 | 22 | 45 | 52 | 43 | 58 | 39 |
| Dose 3 | | | **n = 181** | **n = 183** | **n = 186** | **n = 179** | **n = 181** | **n = 180** | **n = 177** |
| | Fever (%) | ≥ 38.5°– < 39 °C | 24 | 6 | 16 | 20 | 15 | 21 | 7 |
| | | 39.0°– < 39.5 °C | 4 | 2 | 3 | 8 | 5 | 6 | 0 |
| | | 39.5°– < 40.0 °C | 3 | 1 | 1 | 2 | 3 | 3 | 1 |
| | | ≥ 40.0 °C | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| | Medically attended fever (n) | | 0 | 2 | 1 | 1 | 2 | 2 | 1 |
| | Antipyretic use (%) | | 36 | 14 | 28 | 34 | 27 | 35 | 20 |
| Dose 4 | | | **n = 155** | **n = 168** | **n = 169** | **n = 162** | **n = 168** | **n = 165** | **n = 162** |
| | Fever (%) | ≥ 38.5°– < 39 °C | 33 | 18 | 21 | 31 | 29 | 24 | 30 |
| | | 39.0°– < 39.5 °C | 16 | 4 | 11 | 16 | 13 | 14 | 15 |
| | | 39.5°– < 40.0 °C | 3 | 4 | 4 | 6 | 4 | 3 | 4 |
| | | ≥ 40.0 °C | 1 | 0 | 2 | 1 | 1 | 1 | 1 |
| | Medically attended fever (n) | | 1 | 2 | 3 | 1 | 1 | 3 | 4 |
| | Antipyretic use (%) | | 54 | 25 | 49 | 51 | 45 | 57 | 92 |




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### Authors' contributions

SE, RP, GVZ, MB, PD and DT participated in the conception and design of the trials. SE, RP and GVZ managed study sites and enrolled participants. DT, MB and PD performed study management for the study sponsor, FX performed all statistical operations. All authors were involved in the interpretation of analyzed data and the decision to submit for publication, and commented and developed the manuscript from the initial draft.


### Funding statement

The study was fully financed by Novartis Vaccines and Diagnostics. The study sponsor designed the study with the study investigators, drafted the protocol, supplied all materials, analyzed sera, collated data and performed all statistical analyses.


### Supplementary Materials

Supplementary materials may be found here:
www.landesbioscience.com/journals/vaccines/article/29218

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